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Data Sheet
TDO Primer Mix-qPCR Assay
Catalog: # 71271

Description	Primer mix for amplifying human TDO cDNA using dye-based, real time, quantitative PCR (RT-qPCR)
Gene Description	<i>Homo sapiens</i> tryptophan 2,3-dioxygenase (TDO), mRNA.
Gene Synonym	TDO, TDO2, TRPO, TPH2
NCBI GeneID	6999
GenBank Accession	NM_005651
Uniprot	P48775
Species	Human
Coding DNA Length	1221
Assay Type	SYBR® Green
Amplicon Size (bp)	110
Amplicon Sequence	TCCTCAGGCTATCACTACCTGCGATCAACTGTGAGTGATAGG TACAAGGTATTTGTAGATTTATTTAATCTTTCAACATACCTGAT TCCCCGACACTGGATACCGAAGAT
Assay Design	Intron-spanning
Purification	Desalted
Efficiency (%)	86.0
cDNA Tm	75.5°C
Real-Time PCR Instrument	CFX Connect Real-Time PCR Detection System
Real-Time PCR Supermix	iTaq™ Universal SYBR® Green Supermix (Bio-Rad, cat. #1725120)
Materials Required but not Included	SYBR Mix, PCR machine capable of real time PCR measurements

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Storage/Stability -20°C for up to 1 year when stored as supplied; avoid freeze/thaw cycles.

Generalized Assay Protocol

The following procedure is designed to use iTaq™ Universal SYBR® Green Supermix in a 10 µl reaction. To use the qPCR reagent in different formats, follow the manufacturer's recommended assay protocol. The concentration of cDNA sample and qPCR primers should be optimized according to the sample quality and study requirements.

All amounts and volumes in the following setup are provided on a per reaction basis.
Note: we recommend setting up each condition in at least triplicate, and preparing a reaction master mix for multiple wells.

1. Thaw iTaq™ universal SYBR® supermix and other frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solutions at the bottom of tubes, then store on ice, protected from light.
2. Prepare (on ice or at room temperature) enough assay master mix for all reaction by adding all required components, except the DNA template. For one reaction, use 5 µl SYBR® 2x supermix and 1 µl 10x TDO qPCR primer mix (3 µM 10x primer mix would give a final concentration of 300 nM each primer. Usually, 300 nM -500 nM final concentration of each primer is optimal for qPCR reaction.) and the variable amount of TCEP-treated DNase-free H₂O to add up to 10 µl total reaction volume, including DNA template to be added in the end.

Note: Usually, 100 ng-100 fg of cDNA or 50 ng-5 pg of genomic DNA is used as the DNA template in one qPCR reaction.

Reagent	Amount
SYBR® 2x supermix	5 µl
10x TDO qPCR primer mix	1 µl
TCEP-treated DNase-free water	4 -x µl
cDNA template	x µl
Total	10 µl

3. Mix the assay master mix thoroughly to ensure homogeneity and dispense equal aliquots into each qPCR tub or into the wells of a qPCR plate. Good pipetting technique must be employed to ensure assay precision and accuracy.
4. Add DNA samples to the PCR tubes or wells containing assay master mix, seal tubes or wells with flat caps or optically transparent film, and vortex 10 seconds or more to ensure thorough mixing of the reaction components. Spin the tubes or plate to remove any air bubbles and collect the reaction mixture in the vessel bottom.

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5. Program the thermal cycling protocol on a qPCR instrument according to the following steps: Polymerase activation and DNA denaturation at 95°C, 20-30 seconds for cDNA or 2-5 minutes for genomic DNA; 2-5 seconds of denaturation at 95°C, 15-30 seconds of annealing/extension and plate read at 60°C, repeat 2-5 seconds denaturation, annealing/extension and plate read step for 40 cycles. Perform melt curve analysis at the end by increasing temperature from 65-95°C in 0.5°C increments at 2-5 seconds.
6. Load the PCR tubes or plate into the qPCR instrument and start the PCR run.
7. Perform data analysis according to the instrument-specific instructions.

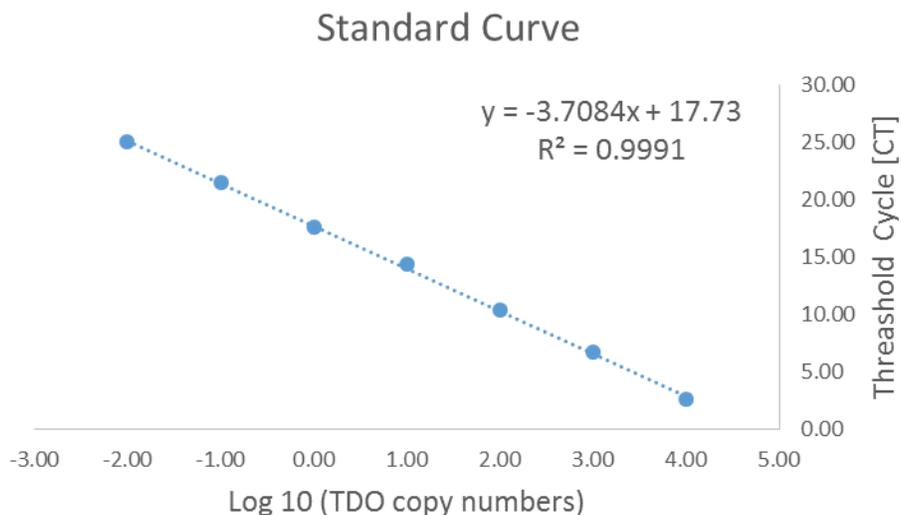


Figure 1. qPCR standard curve generated with known concentration of hTDO cDNA on linearized plasmid. $R^2 = 0.9991$

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